THE Marquardt CORPORATION VAN NUYS, CALIFORNIA

1570142

14 p.

FIFTH MONTHLY LETTER REPORT
1 Mar. - 31 Mar. 1964
(NASA Contract NASw-810)

STUDY OF MARBAC EXTRATERRESTRIAL LIFE DETECTION CONCEPT

For Period of 1 March through 31 March 1964

Prepared for: Chief of Exobiology, Bioscience Programs
Office of Space Sciences
National Aeronautics and Space Administration
Washington 25, D.C. 20546

Prepared by:

E. E. Sweeney
Principal Investigator

and

G. E. Ellis
Electrochemist

[1964] 14p ea

Checked by:

J. G. Bitterly
Principal Research Scientist
Bioastronautics

Approved by:

A. N. Thomas
Chief Research Scientist
Advanced Projects

Enclosure (1)
LR 341-5

April 1964

The following summarizes last month's work in the microbiological research program to establish the usefulness of oxidation-reduction potential change for extraterrestrial life detection.

19577

SUMMARY

A

Bacterial growth, primarily cell division, was studied with relation to oxidation-reduction potential change. Relatively large inocula were used (e.g., 10^7 organisms/milliliter). The rate of change in potential correlated in general with increase in cell numbers; the rate of change of potential reached its maximum at approximately the time the cell growth reached its maximum rate. Correlation among viable counts (plating), Coulter counts, and turbidimetric readings indicated that with large bacterial concentrations each method could be useful in redox investigation.

Author

I DISCUSSION

A. Laboratory Experiments

Escherichia coli, as a model organism for most experiments, was grown in rich test nutrient with a heavy inoculum in order to provide relatively rapid large potential changes. Bacterial count densities were determined by plating, Coulter Counter, and turbidimetry. Techniques were discussed in the previous month's report.

Results of the experiments on evaluation of bacterial counting procedures are shown in Figures 1 to 4 (Bacillus subtilis was used in Experiment 59, Figure 4, rather than E. coli). Plate and Coulter counts were averaged from four tests at each concentration. Typically, three dilutions of a serial set were tested, e.g., 10^{-5}, 10^{-6}, and 10^{-7}. Plates were read which contained 30 to 300 colonies.

In general, potential changes were proportional to bacterial counts. However, there appears to be a tendency for potential to change sooner than bacterial count. This observation is in keeping with the established fact that bacteria are typically very active metabolically just prior to entering a logarithmic growth phase.

A number of new species were tested for redox changes in a single routine complex growth medium, trypticase soy broth (Baltimore Biological Laboratory, Inc.). This work was done primarily to establish redox techniques, to understand general handling problems related to organisms other than E. coli, and to evaluate reproducibility. Results are shown in Figures 5 to 7. Each curve shown in a figure was averaged from three tests with each organism. Differences between curves of distinct species will undoubtedly correlate to different biochemical pathways and metabolic rates between the microbes. Our future experimental work will attempt to analyze the differences.

An attempt was made to relate variation among the potential changes of three test flasks of one specific microbe to variation in cell counts, by Coulter procedure (Experiment 60, Figure 7). However, potential and count changes were very similar for the three tests of Bacillus megaterium and of Serratia marcescens; variations of potential seemed to be within the limits of experimental error. Thus, Figure 8 demonstrates two of the Serratia potential curves with Coulter counts at several intervals. The reproducibility of potential (and of counts) is a considerable improvement over earlier work. The 40 millivolt

difference between the curves at 12 hours could not be analyzed because no bacterial counts were made at that time. Streptococcus faecalis showed relatively wide variation of potential change, among three tests; these changes did not consistently correspond to changes in Coulter counts.

B. Laboratory Techniques and Instrumentation

The Coulter Counter is presently being applied to much of the research program. Thus far, the instrument has been used only in counting particles rather than in sizing. The current counting technique is already a valuable adjunct to the MARBAC program, but still may be improved considerably with regard to time required per test.

For example, difficulties have been experienced in the clogging of the 30 micron aperture through which the suspension flows when its electrolytic conductivity is being measured in the Coulter Counter. This problem may be solved when new fused manometers arrive, which allow cleaning by rigorous methods not permissible with the cemented orifices now being used. Application of detergents to the solution used to dilute the bacterial suspension before counting is being investigated. Detergents may: (1) decrease aperture clogging, (2) decrease bacterial clumping or increase dispersion, and (3) decrease bacterial conductivity as an aid to counting.

Studies have been initiated to use the Coulter Counter to size particles, since it is well established that a bacterial species can vary considerably in size in different growth phases or conditions. Information regarding particle size or electrical surface area is necessary, since particle counts alone may not provide information which could accurately correlate metabolism rates with potential changes.

New company-purchased instrumentation has been ordered to satisfy requirements established in previous months' efforts. A Dymec Model 2010F-M5 automatic IBM card recording system will shortly be installed in the microbiological laboratory which will present an input impedance of not less than 1000 megohms for potential readings to $\pm$0.1 millivolt; the present automatic recording digital voltmeter has an input impedance of 10 megohms. The new automatic data sampling time for each test cell can be selected down to 0.1 second, compared to the three seconds required in the present system. With the Dymec system potential readings can be taken every five seconds for 50 channels (or less time for fewer channels), compared to the present 30 minute interval between potential readings.

II WORK PLANNED FOR ENSUING MONTH

A. Start of analysis and testing of media specific for vigorous growth of each microbial species tested

B. Application of statistical analysis to bacterial counts

C. Correlation of polarographic analyses to redox potential studies

TABLE I

CONSTITUENTS OF TRYPTICASE SOY BROTH
(Baltimore Biological Laboratory, Inc.)

INGREDIENTS	GRAMS/LITER
Trypticase	17.0
Phytone	3.0
Sodium chloride	5.0
Dipotassium phosphate	2.5
Dextrose	2.5

CHANGES IN POTENTIAL AND VIABLE COUNT BY E. COLI
IN TRYPTICASE SOY BROTH*
EXPERIMENT NO. 55

INOCULUM: E. COLI, 18 hr CULTURE GROWN AT 37°C

TEST CONDITIONS:
TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - = UNINOCULATED
——— = INOCULATED

TESTS:
○ = POTENTIAL
□ = VIABLE COUNT (PLATE)

*DESCRIBED IN TABLE I

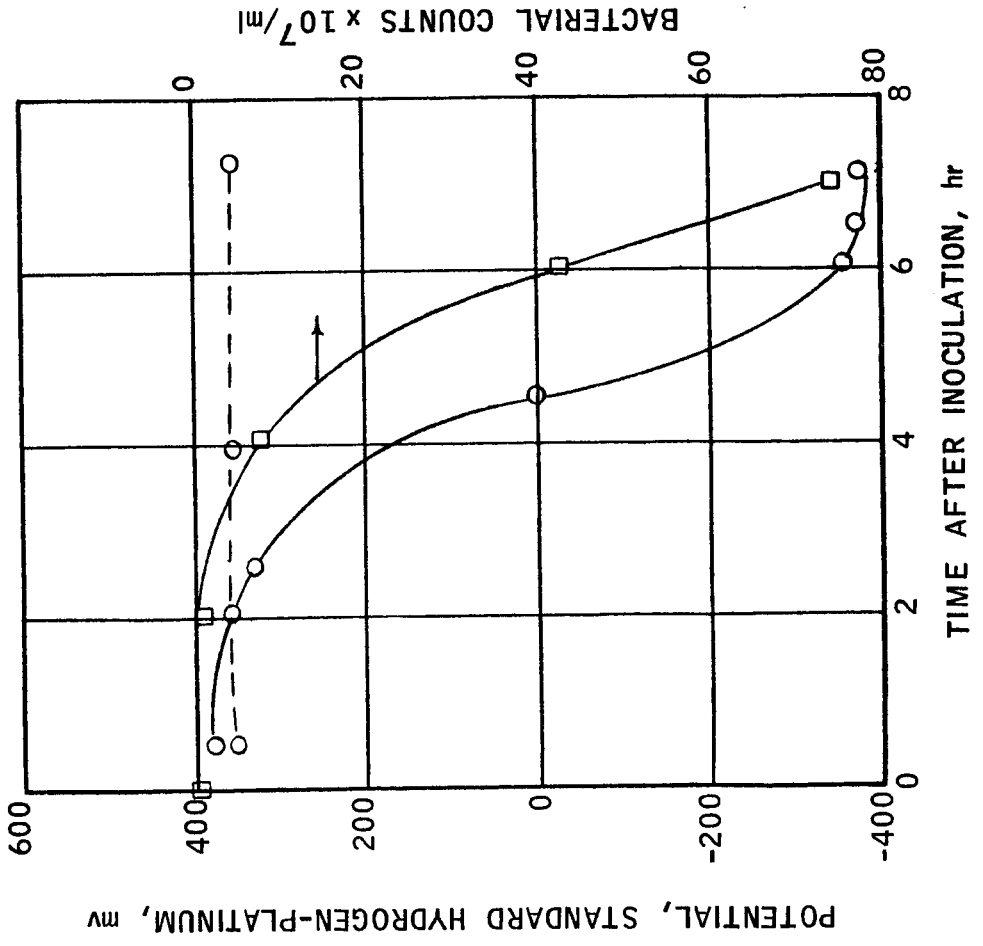


FIGURE 1

CHANGES IN POTENTIAL, VIABLE COUNT, AND COULTER COUNT BY *E. COLI* IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 56

INOCULUM: *E. COLI*, 18 hr CULTURE GROWN AT 37°C

TEST CONDITIONS:
TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

TESTS:
○ = POTENTIAL
□ = COULTER COUNTS
◇ = VIABLE COUNTS

*DESCRIBED IN TABLE I

- - - - = UNINOCULATED
——— = INOCULATED

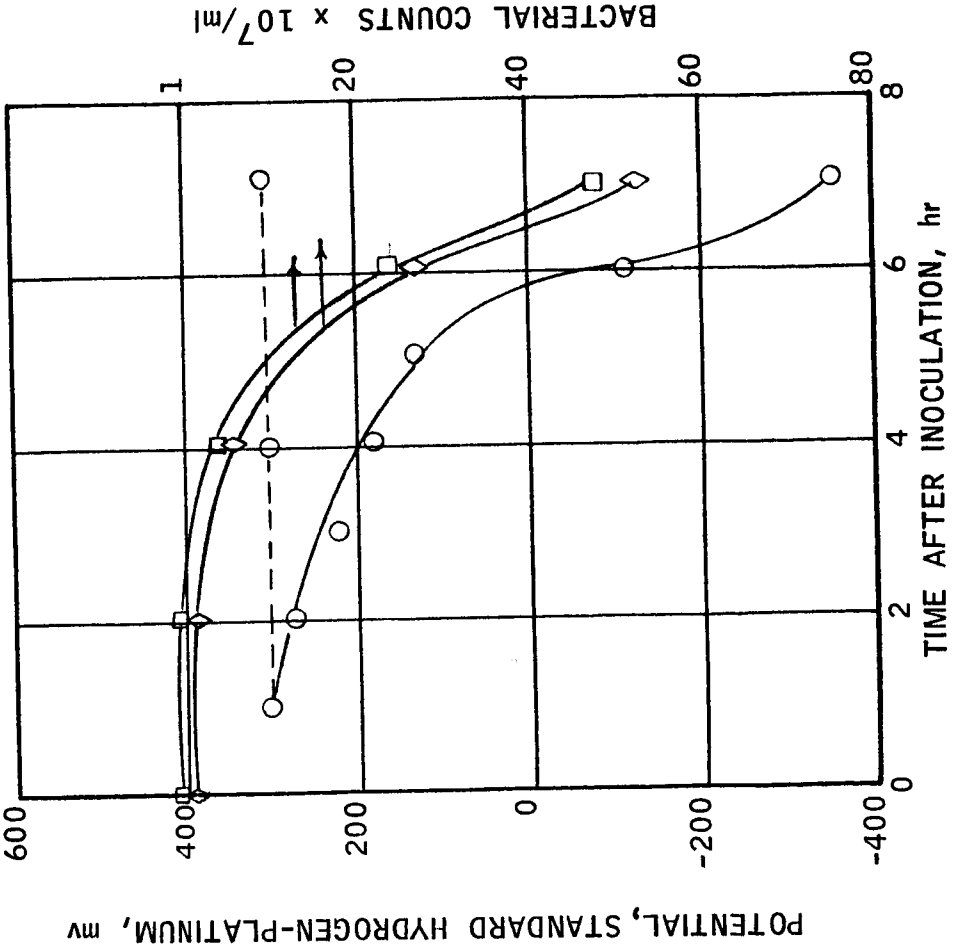


FIGURE 2

CHANGES IN POTENTIAL AND OPTICAL DENSITY BY E. COLI IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 56

INOCULUM: E. COLI, 18 hr CULTURE GROWN AT 37°C
INOCULUM SIZE: APPROX. 10^7 ORGANISMS/ml

TEST CONDITIONS:
TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - = UNINOCULATED
——— = INOCULATED

TESTS:
O = POTENTIAL
□ = OPTICAL DENSITY

*DESCRIBED IN TABLE I

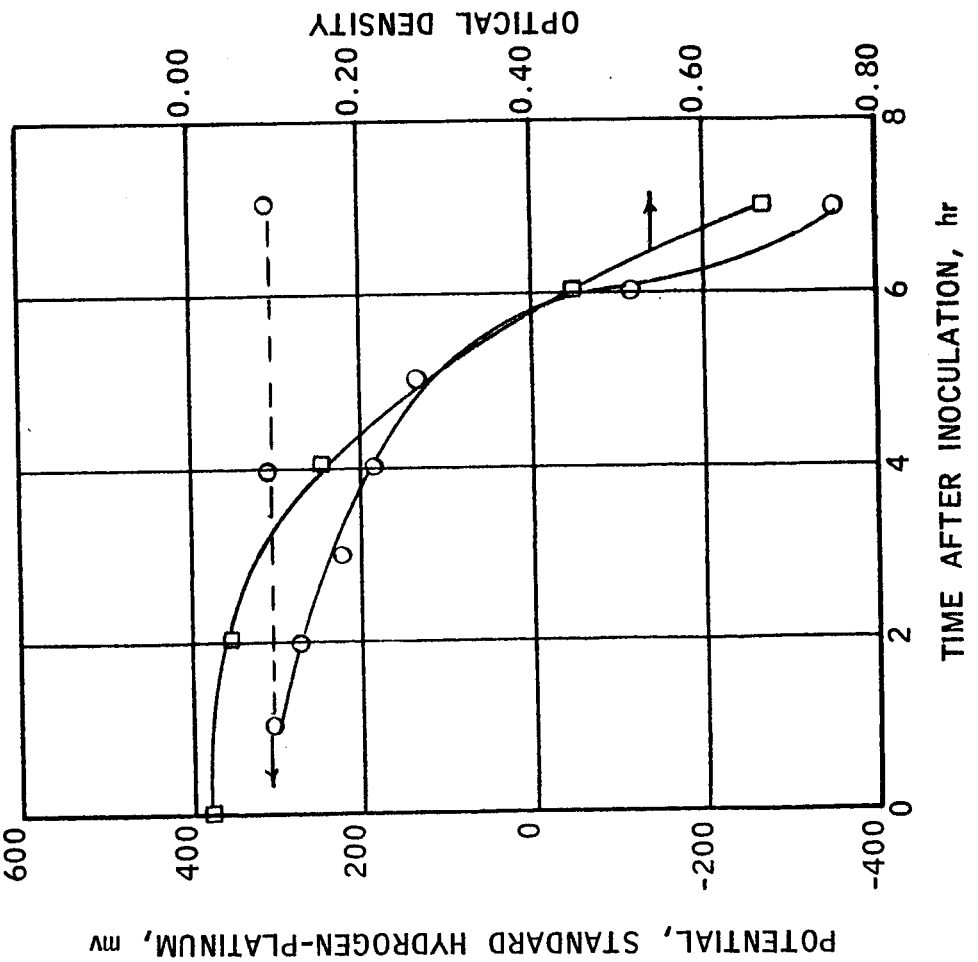


FIGURE 3

CHANGES IN POTENTIAL AND VIABLE COUNT BY B. SUBTILIS IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 59

INOCULUM: B. SUBTILIS, 18 hr CULTURE GROWN AT 37°C

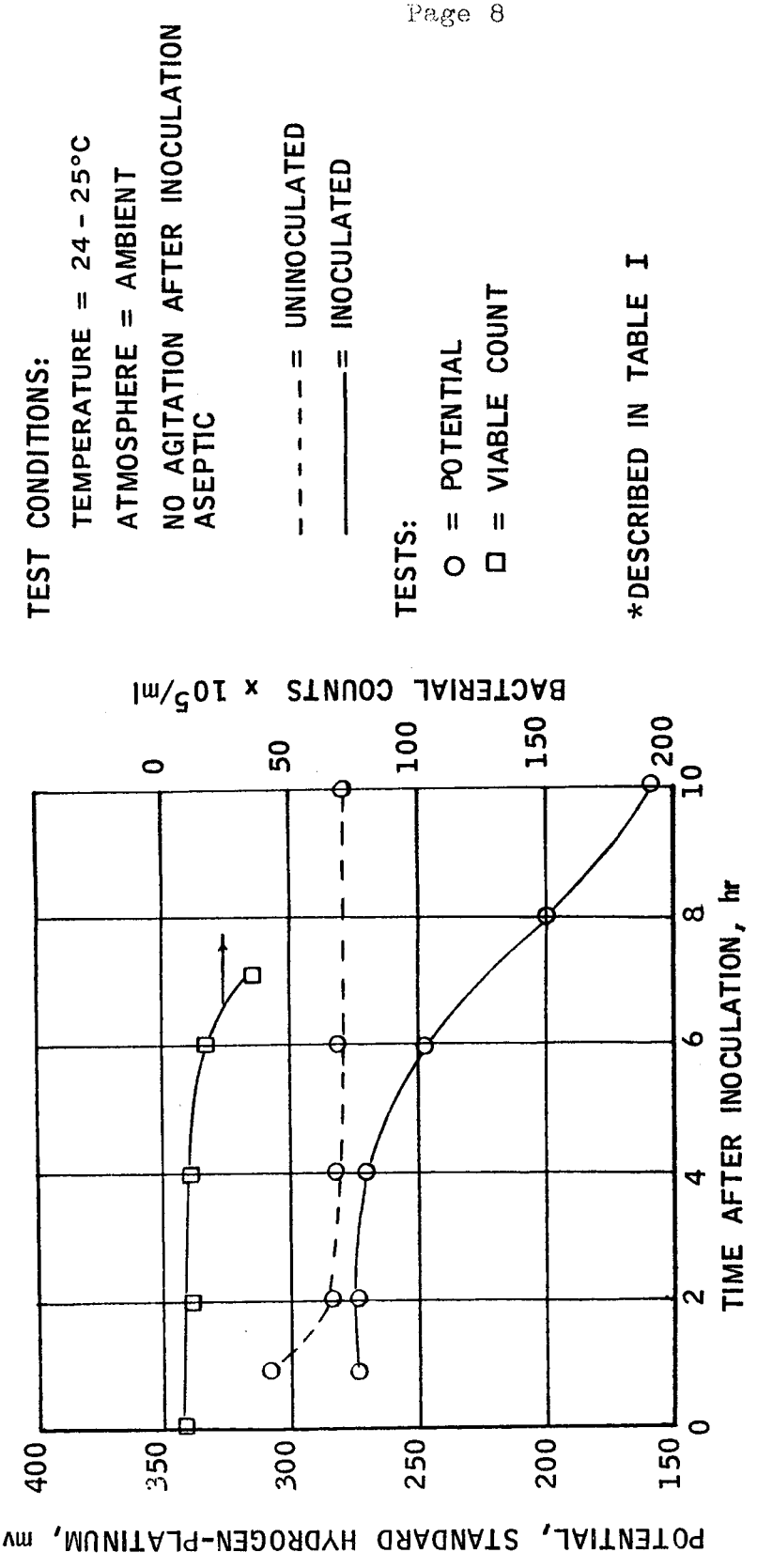


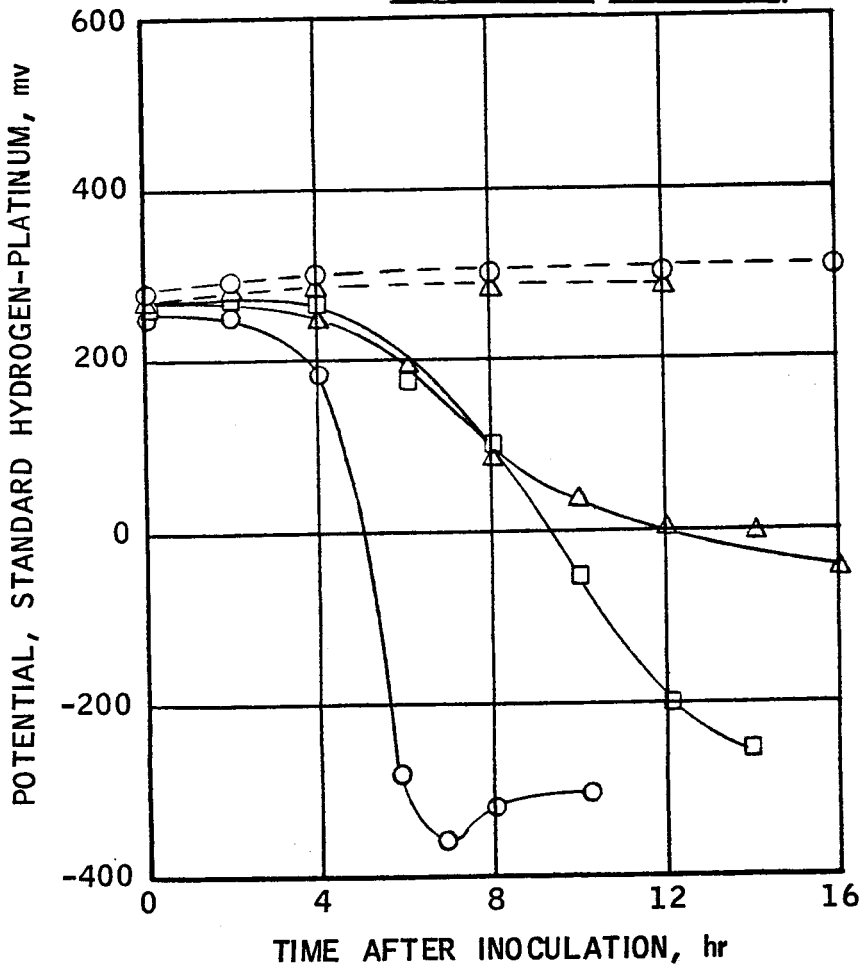
FIGURE 4

CHANGES OF POTENTIAL PRODUCED BY A. FAECALIS, E. CARATOVORA, AND A. AEROGENES IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 57

INOCULA: ALL 24 hr CULTURES GROWN AT 37°C

INOCULA SIZES: ALCALIGENES FAECALIS, APPROX. 10^8 ORGANISMS IN 31 ml
ERWINIA CARATOVORA, APPROX. 10^8 ORGANISMS IN 31 ml
AEROBACTER AEROGENES, APPROX. 10^9 ORGANISMS IN 31 ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

– – – – – – = UNINOCULATED

——————— = INOCULATED

TESTS:

△ = ALCALIGENES FAECALIS

□ = ERWINIA CARATOVORA

○ = AEROBACTER AEROGENES

*DESCRIBED IN TABLE I

CHANGES OF POTENTIAL PRODUCED BY B. CEREUS, B. SUBTILIS, AND P. FLUORESCENS IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 58

INOCULA: ALL 23 hr CULTURES GROWN AT 37°C
INOCULA SIZES: ALL INOCULA APPROX. 10^8 ORGANISMS IN 31 ml

TEST CONDITIONS:
TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - UNINOCULATED
——— INOCULATED

TESTS:
○ = BACILLUS CEREUS
□ = BACILLUS SUBTILIS
△ = PSEUDOMONAS FLUORESCENS

*DESCRIBED IN TABLE I

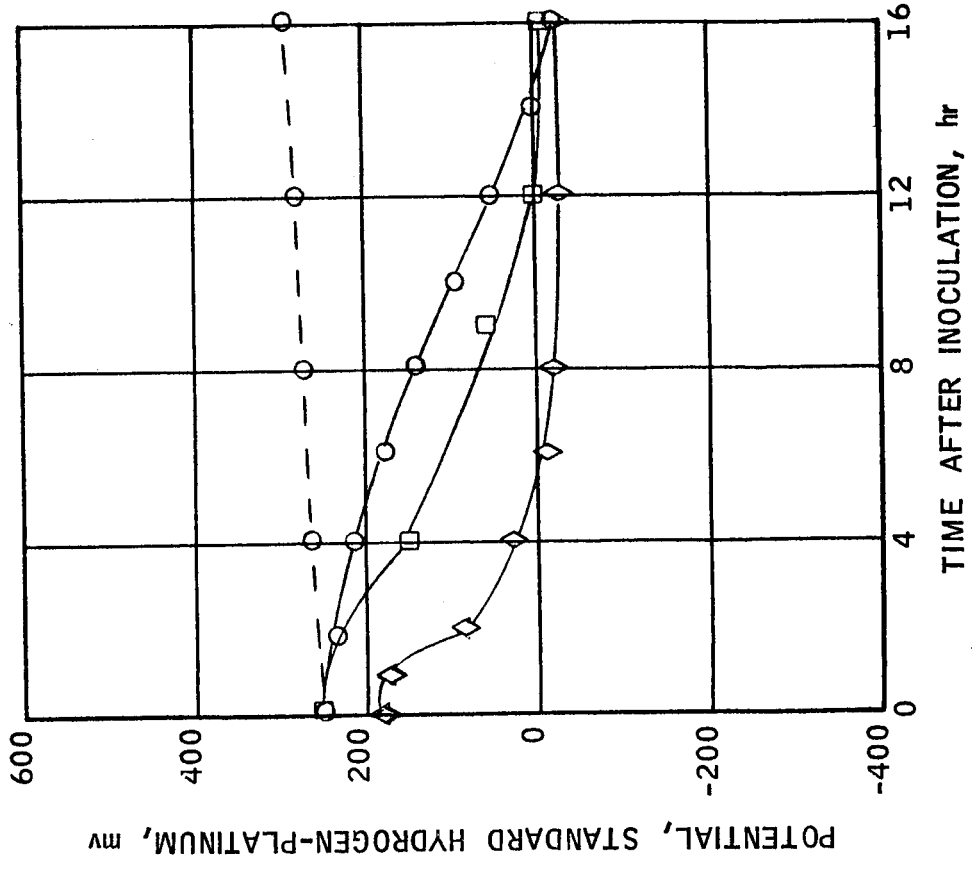


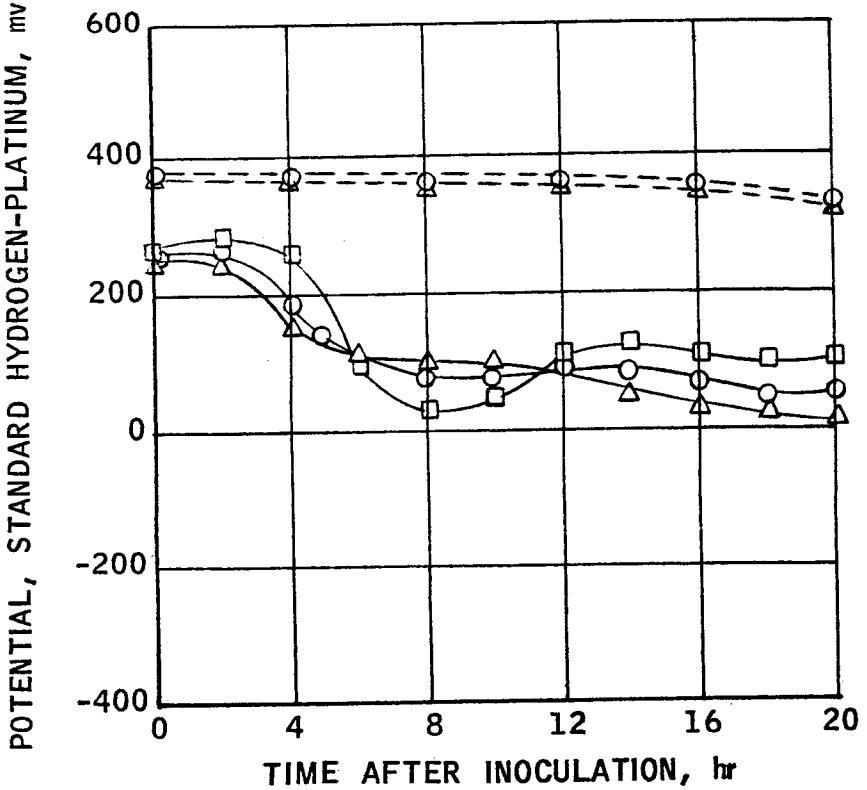
FIGURE 6

CHANGES OF POTENTIAL PRODUCED BY B. MEGATERIUM, S. FAECALIS, AND S. MARCESCENS IN TRYPTICASE SOY BROTH*

EXPERIMENT NO. 60

INOCULA: ALL 18 hr CULTURES GROWN AT 37°C

INOCULA SIZES: BACILLUS MEGATERIUM, APPROX. 5×10^6 ORGANISMS/ml
STREPTOCOCCUS FAECALIS, APPROX. 10^7 ORGANISMS/ml
SERRATIA MARCESCENS, APPROX. 10^7 ORGANISMS/ml



TEST CONDITIONS:

TEMPERATURE = 24 - 25°C

ATMOSPHERE = AMBIENT

NO AGITATION AFTER INOCULATION
ASEPTIC

- - - - - - - = UNINOCULATED

——————— = INOCULATED

TESTS:

○ = BACILLUS MEGATERIUM

□ = STREPTOCOCCUS FAECALIS

△ = SERRATIA MARCESCENS

*DESCRIBED IN TABLE I

FIGURE 7

REDOX REPRODUCIBILITY COMPARED TO BACTERIAL COULTER COUNTS

EXPERIMENT NO. 60

MEDIUM: TRYPTICASE SOY BROTH*

INOCULUM: SERRATIA MARCESCENS, 18 hr CULTURE GROWN AT 37°C

TEST CONDITIONS:
TEMPERATURE = 24 - 25°C
ATMOSPHERE = AMBIENT
NO AGITATION AFTER INOCULATION
ASEPTIC

TESTS:
O = FLASK 24
Δ = FLASK 26
(FLASKS 24 AND 26 WERE DUPLICATE TESTS)

*DESCRIBED IN TABLE I

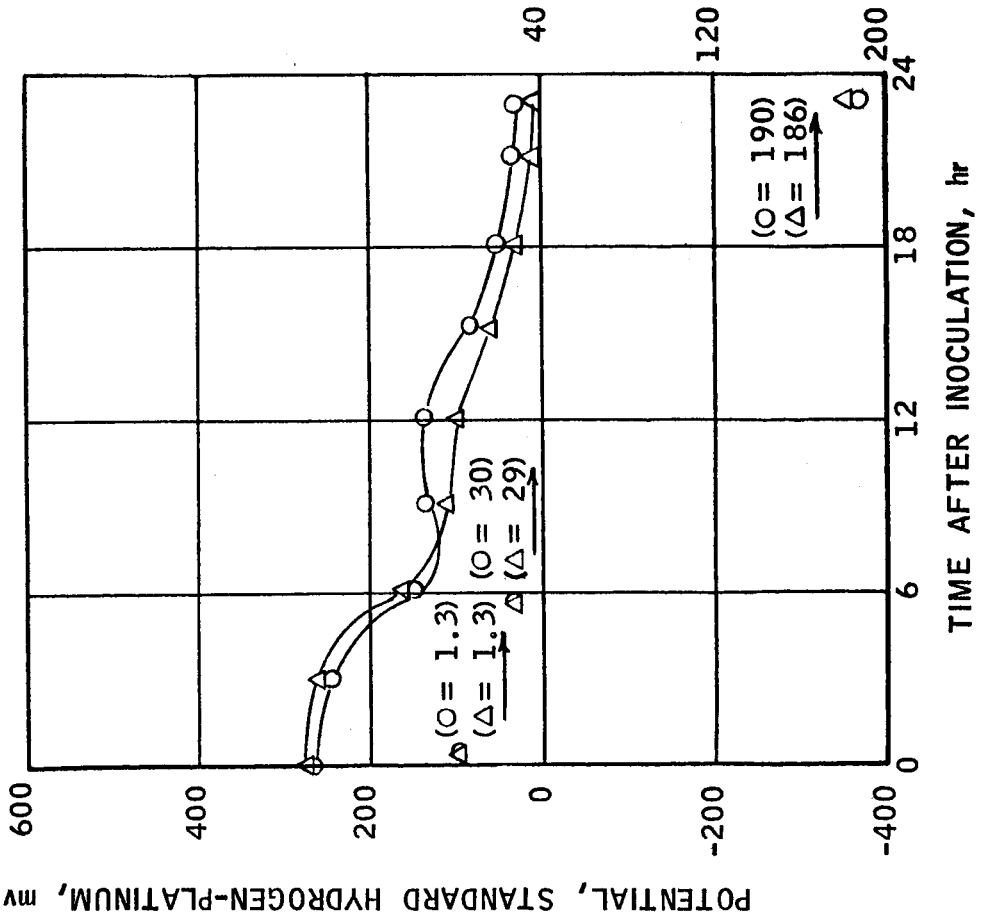


FIGURE 8